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THE BIOLOGICAL FUNCTION OF ANTIMURINE SPERM-SPECIFIC LACTATE DEHYDROGENASE C4 MONOCLONAL IGA ASSEMBLED WITH OR WITHOUT SECRETORY COMPONENT

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Abstract In order to determine the biological role of antisperm IgA, especially secretory IgA, in immunological infertility and in the development of an antisperm contraceptive vaccine, a panel of monoclonal IgA (moIgA) antibodies to lactate dehydrogenase C4 (LDH-C4) were prepared by intraintestinal immunization, and their heterogeneous isoforms were demonstrated by Western blotting. Most moIgAs (PA1-PA5) were obtained by the combined use of intraintestinal immunization and fusion of Peyer's patch cells for parent cells. In the presence of guinea pig serum complement, mouse sperm were immobilized by moIgA PA1, PA2 and PA4. Mouse sperm were agglutinated by PA4 and PA5 at high concentration. The rates of *in vitro* fertilization were significantly reduced by 3 of 7 moIgA tested, but moIgA (PA1, PA2 and PA5) had no significant effects on mouse *in vivo* fertilization by passive immunization. Purified murine bile secretory component was assembled with either

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purified moIgA or moIgA in ascites *in vitro*. The effects of moIgA on sperm immobilization, sperm agglutination and *in vitro* fertilization were not significantly changed following the binding of the moIgA with secretory component. These results provide direct evidence of a positive biological role of anti-LDH-C4 moIgA and secretory IgA in sperm function and *in vitro* fertilization.

Key words Monoclonal IgA, Lactate dehydrogenase C4, Sperm immobilization and agglutination, *In vitro* fertilization, Secretory component

1 INTRODUCTION

Several antihuman sperm contraceptive vaccine candidates have been selected by World Health Organization-sponsored Workshops (Anderson *et al.*, 1987). Among them, the subsequent cloning and sequencing of cDNA coding for SP-10 and MSA-63 have been reported (Wright *et al.*, 1990; Liu *et al.*, 1990). Lactate dehydrogenase C4 (LDH-C4) is the first well-defined sperm-specific protein antigen. A cDNA clone of human LDH-C4 was identified and sequenced (Millan *et al.*, 1987). Monkey and human tissue cultured cells infected with the purified human ldh-c recombinant vaccinia expressed the enzymatically active tetramer. Rabbits immunized with the recombinant vaccinia produced antibodies to human LDH-C4 (LeVan and Goldberg, 1989). A significant reduction in fertility was observed in female mice, rabbits and baboons immunized with purified LDH-C4, but the contraceptive effect is not complete and not well correlated with the level of serum antibodies in systematically immunized animals. After intrauterine immunization with LDH-C4, secretory IgA to LDH-C4 in mouse uterine fluids was detectable, and the immunized animals demonstrated a lower pregnancy rate than controls (Shelton and Goldberg, 1986).

In mammals, gamete maturation and transportation, binding and fusion between egg and sperm are conducted in the genital tracts, in which secretory IgA composed of polymer IgA and secretory component (SC) is a major immunoglobulin. With respect to immunological infertility and development of a contraceptive vaccine, it is well-known that many monoclonal antisperm IgG and IgM are able to agglutinate and immobilize sperm and to inhibit mouse fertilization *in vitro* and *in vivo* (Anderson *et al.*, 1987; Saling and Waibel, 1985; Ben *et al.*, 1988). The biological role of IgA, especially secretory IgA, is not clear because it is impossible to collect and to purify sufficient sperm antigen specific IgA from mucosal secretions of human and experimental animals for function tests. In this report, the monoclonal IgA (moIgA) to murine LDH-C4 has been obtained by a special immunization technique, and the biological function of the moIgA assembled with or without SC was investigated.

2 MATERIALS AND METHODS

Animals. Male and female Balb / c mice and Swiss Webster outbred mice were bred and housed in the animal breeding facilities of Kunming Institute of Zoology.

LDH-C4 isolation and purification. LDH-C4 was isolated and purified as described by Wang *et al.* (1990). Briefly, the testes of sexually mature mice were homogenized, heated in a water bath at 65℃ for 15 min, and then cooled in an ice-bath. After centrifugation at 15000 rpm for 20 min at 4℃, the supernatant was loaded onto 5′-AMP-Sepharose 4B column. The fractions with high enzyme activities were collected, concentrated and then passed through a Sephadex A50 gel column. After concentrated, the first peak was placed onto a DEAE-Sepharose CL-6B column. The LDH-C4 in the fractions of first peak was collected and concentrated.

Immunization. For preparation of positive antisera to LDH- C4, immunization procedures were used by the method described by Kille *et al.* (1978). For hybridoma production, the immunization procedures were adopted from Weltzin *et al.* (1989). The protocols are summarized in Table 1. For injection into the Peyer's patch (PP) submucosa, the peritoneal cavity was opened by carefully making a cut of 5 mm length of the mid-left peritoneal wall, and the small intestine was pulled out of the cavity. About 20 μl of LDH-C4 solution (0.5 mg / ml) was injected into each of 5 or more PP by using a 30G1 / 2 needle (Becton Dickinson, Rutherford, New Jersey). Appearance of a pale spot on PP confirmed the injection to be all right. For intra-luminal immunization, 100 μl of LDH-C4 solution was injected into the lumen of the proximal duodenum with a 30G1 / 2 needle. After these immunizations, the wound should be sewed with fine thread.

Cell isolation. For isolation of cells from PP, all of PP were very carefully removed by using fine scissors, and washed three times with RPMI 1640 containing 50 μg gentamycin, 100 units penicillin and streptomycin per ml of medium. The PPs were then ground by ends of two sterile frosted-end microscope slides or homogenized with small glass grinder. After allowing large debris to settle for 5 min, the supernatant was collected and cells were then washed twice by centrifugation at 200 ×g for 5 min with complete medium containing RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY), 10% newborn bovine serum (NBS), 2 mM glutamine, 10 mM HEPES, 100 units penicillin and streptomycin, 10 μg gentamycin per ml. The spleen cells were isolated by using the similar technique. The cell viability was greater than 90% by trypan blue exclusion.

Table 1 Immunization protocols

Days	Route	Dose(μg)
0	i. pp	50
14	i. l.	50
21	i. p.	50
24	cell fusion	

abbreviations: i. pp., antigen injected into Peyer's patch submucosa; i. l., antigen injected into the intestinal lumen; i. p., antigen injected intraperitoneally.

Myeloma cells and fusion. NS-1 cells were grown in complete medium. NS-1 cells and lymphocytes from immunized mice were washed twice with serum-free RPMI 1640 medium, then mixed and fused in 50% PEG (MW 1450, Fluka Chemical Corporation, Ronkonkoma, NY) at a leukocyte to NS-1 cell ratio of 1 : 1 for PP or 10 : 1 for spleen. After fusion, the cells were diluted to 1×10^6 cells / ml in complete medium containing 20% NBS, 0.1 mM hypoxanthine (Sigma), 0.4 μM aminopterin (Fluka), 16 μM thymidine (Sigma)(HAT medium), and distributed in 0.1 ml per well to 96-well tissue culture plates (for fusion of PP cells) and in 1 ml per well to 24-well plates (for fusion of spleen cells). These tissue culture plates had been seeded 24 h earlier with mouse thymocytes or peritoneal exudate cells. One week later, 1.0 ml of HAT medium was removed from each well of 24-well plates, and replaced with the same amount of HAT medium. For the 96-well plate, 0.1 ml of HAT medium was added directly to each well.

Screening of hybridomas by the enzyme-linked immunosorbent assay (ELISA). An indirect ELISA was used to screen for antibodies to LDH-C4. One hundred μl of LDH-C4 in 0.01 M, pH 7.5 PBS was coated on 55-well microtiter plate overnight at 4℃. After washing 3 times with 0.05% Tween-saline, the plate was blocked with 0.3% gelatin-PBS at 4℃ for 1 h. One hundred μl of supernatant from each tissue culture plate well with hybridoma was added to each microtiter well, and the plate was incubated at 4℃ overnight. The peroxidase conjugated goat anti-mouse IgA (alpha chain specific) (Sigma) at dilution of 1 : 500 and peroxidase conjugated rabbit anti-mouse immunoglobulin (Beijing Institute of Biological Products, Beijing) at dilution of 1 : 80 were simultaneously used for the screening of IgA and other antibodies, respectively. The microplates were incubated at room temperature for 1 h. The enzyme substrate consisting of 2, 2′- azino- di(3-ethyl- benzothiazolin-sulfonic acid) (ABTS) and 0.03% hydrogen peroxide in citrate phosphate buffer (pH 4.0) was then added. After 30 min, the reaction was stopped by adding 50 μl of 0.1% sodium azide to each well. A positive reaction was assessed with an EIA reader (Bio-Tek, Burlington, VT) at a wavelength of 405 nm.

Production of monoclonal antibodies. The monoclonal antibody-producing hybridomas were expanded, adapted to complete medium and cloned by limiting dilution. Ascites fluids containing specific antibody were produced by intraperitoneal injection of 2.5×10^6 hybridoma cells into each Balb / c mouse primed with pristane (Sigma).

Detection of subclass of monoclonal antibodies. The immunoglobulin subclass was determined by double diffusion. Rabbit anti-mouse IgG1, G2a, G2b, G3 and IgM (heavy chain specific) were purchased from Miles Laboratory, Elkhart, IN, and goat anti-mouse IgA (alpha chain specific) was from Biomedical, Malvern, PA.

Purification of monoclonal IgA. The ascites was clarified by centrifugation at 9000 rpm for 30 min at 4℃. The supernatant was collected and dialyzed against

0.01 M, pH 7.4 Tris buffer, and then passed through a Sephadex G200 column, which was eluted with the same buffer. The fractions in peak 1 were pooled and applied to a DEAE-Cellulose 32 column. The column was eluted with 0.01 M pH7.4 PBS, and the first peak was discarded. Thereafter, the column was eluted with 0.145 M NaCl in 0.01 M PBS, and the fractions in the peak containing IgA were collected.

Determination of IgA polymers. Immunoblotting was performed to identify IgA polymer (Weltzin *et al.*, 1989). The purified moIgA, IgA-containing ascites fluids and molecular weight standards (45-480 kDa) (Sigma) were run on nonreducing 3%-10% gradient gel, and then transferred to Transphor nitrocellulose membrane (Hoefer Scientific, San Francisco, CA). The samples were immunostained with peroxidase-conjugated goat anti-mouse IgA (Sigma). DAB (3, 3′-diaminobenzidine) and 4- chloro -1-naphthol (Sigma) were used as substrates (Young *et al.*, 1989).

Isolation and purification of SC. Collected mouse bile was diluted with an equal volume of 0.1 M Tris buffer (pH 7.6) containing 0.5 M NaCl, 1 mM phenyl methyl sulfonyl fluoride (PMSF), 50 mM 6-amino n-caproic acid, 0.1% NaN_3 and 50 mM EDTA, and centrifuged at 7000 rpm, 4℃ for 1 h. After removing the layer of fat, the supernatant was applied to a Sephadex G200 column. After eluting with 0.1 M Tris buffer, the fem polysulfone filter (nominal molecular weight limit 10000) (Millipore, Bedford, MA). The concentrate was desalted by on a filtration through Sephadex G25 column in 0.01 M PBS (pH 7.4), and chromatographed on a DEAE Cellulose 32 column. Following elution with 0.01 M PBS, fractions containing pure SC were obtained by eluting with 0.145 M NaCl in 0.01 M PBS. The purity of SC was determined by SDS-PAGE.

Assembly of moIgA with SC. The detailed procedures have been described elsewhere (Chen *et al.*, 1994). Briefly, the purified moIgA or ascites fluid were mixed with purified SC at a molar ratio of 1 : 1, gently shaken at 37℃ for 2 h and incubated overnight at 4℃. In order to confirm that assembly was completed, Western blotting was conducted by the method described above. The nondenatured gradient PAGE of samples tested was performed. Rabbit anti-purified human SC serum was prepared in this laboratory and the reaction of the antiserum with mouse SC, but not with purified moIgA was observed by using double diffusion. Rabbit anti-human SC serum at a dilution of 1 : 100 in 0.1% defatted milk powder was added to secretory-IgA-transferred nitrocellulose and incubated at 4℃ for 8 h. The peroxidase- coupled mouse anti-rabbit IgG monoclonal antibody (Biotechnology Center of Chinese Ministry of Agriculture, Beijing) at 1 : 500 dilution was then reacted at 37℃ for 1 h. The same substrates described above were used for color development.

Sperm agglutination (SA) and immobilization (SI) assays. The modified microassays were carried out for the evaluation of effects of the moIgA on mouse sperm function

(Rose *et al.*, 1976). Briefly, mouse sperm was obtained by epididymal flushing and the sperm concentration was adjusted to 2×10^7 / ml with modified Krebs Ringer bicarbonate medium (MKRB). The monoclonal antibodies, mouse anti-LDH-C4 serum (positive control), NS-1 ascites fluid (negative control) and sperm suspension (blank control) as well as normal guinea pig serum (control for complement) were all diluted with MKRB. For the SA assay, 30 μl MKRB was added to each well, and 30 μl of samples at serial double dilution were subsequently added. Five μl of sperm suspension and 2 drops of paraffin oil were successively distributed to each well. The plates were incubated in moisture saturated 5% CO_2 / air at 37℃ for 2 h. The results were evaluated on an inverted microscope. Similar procedures were used for the SI assay. Thirty μl of guinea pig serum containing 64 CH50 unit of complement per ml was added to each well except blank control and inactivated guinea pig serum control. The plates were incubated in moisture saturated 5% CO_2 / air at 32℃ for 1-1.5 h.

In vivo fertilization. Swiss Webster mice 7-8 weeks old were injected intraperitoneally with pregnant mare serum (PMS) (10 IU / mouse) (Changchun Institute of Biological Products, Changchun) and 48 h later with human chorionic gonadotropin (HCG) (10 IU / mouse) (Shanghai Institute of Biochemistry, Shanghai). The mice were given intraperitoneal injections with 0.5 ml of either monoclonal ascites fluid (2-4 mg IgA / ml) or NS-1 ascites fluid or saline 15 min after both the PMS and HCG injections. After the second passive immunization, 1 female mouse was mated with 1 male in an individual cage. About 40-44 h after HCG injection, the oviducts from mice with vaginal copulation plugs after 14-16 h after HCG injection were excised, and ova and embryos were collected from each mouse. The numbers of embryos (2-cell stage) and unfertilized eggs were counted under the inverted phase contrast microscope. Significat differences in fertilization rate between test and control groups were analyzed by two-way chi-square test.

In vitro fertilization. *In vitro* fertilization was conducted by a modified version of the procedure described by Hogan *et al.* (1986). Superovulation of Swiss Webster (4.5-6 weeks old) or C57 / 6J (4-5 weeks old) mice was induced by intraperitoneal injections of 7.5 IU PMS (Sigma) and 7.5 IU HCG (Sigma) administrated 48 h apart. Mouse sperm from epididymis were collected, and the sperm suspension at a concentration of 1.5×10^7 / ml in a modified Whittingham's medium containing 4 mg / ml BSA was prepared. Ten μl of the sperm suspension was added to 290 μl of pregassed modified Whittingham's medium containing 30 μl of either IgA ascites or SC-combined IgA ascites (plus volume of purified SC added before assembly) or NS-1 ascites under paraffin oil (Fisher, 0-121) in an organ tissue culture dish. This drop was incubated at 37℃ in 5% CO_2 / air for 60-90 min. Then, the cumulus masses from one side of the oviducts of 3-4 mice were separately added to an insemination drop for either sample or control groups. The insemination drop containing sperm and eggs was incubated for

8 h, and thereafter examined under the inverted phase contrast microscope. Eggs with two pronuclei or the second polar body were considered to be fertilized. Development of the fertilized eggs to 2-cell embryos could be observed at 22-24 h postinsemination. Statistical analysis was conducted by the same method as on the *in vivo* fertilization assay above.

3 RESULTS

Cells from PP or spleen of mice immunized with LDH-C4 were fused with myeloma cells. The antibodies secreted by hybridomas were screened by ELISA. The hybridomas were cloned by limiting dilution. As shown in Table 2, five moIgA secreting hybridomas (PA1-PA5) were derived from PP, and hybridoma SA1 and SA2 from spleen. In contrast, most cloned hybridomas that secreted IgM were obtained from fusion with spleen cells.

Table 2 Cloned hybridomas secreting antibodies to LDH-C4

Cell source	Number of hybridomas secreting		
	IgA	IgM	IgG
PP	5(PA1-5)*	2(PM1-2)	0
spleen	2(SA1-2)	7(SM1-7)	0

* The codes of monoclonal antibodies are shown in the parentheses.

The ELISA titer of monoclonal IgA in ascites (except PA5) exceeded 15000 (Table 3). Dimer (d), trimer (t) and higher polymer IgA in both supernatant and ascites could be identified by Western blotting in all moIgA listed (Fig. 1). The concentration of total moIgA in ascites ranged from 1.09 mg to 4.45 mg per ml (data not shown) by quantitative ELISA.

Table 3 Mouse sperm agglutination and immobilization caused by monoclonal IgA to LDH-C4

MoAb code	IgA titer① in ascites	Concentration (μg / ml) of IgA inducing significant effect			
		Agglutination		Immobilization	
		A②	B	A	B
PA1	>15725	-	-	8③	7
PA2	>15725	-	-	495	264
PA3	>15725	-	-	-	-
PA4	>15725	430	450	110	245
PA5	3125	1130	-	-	-
SA1	15725	-	-	-	-

① IgA titer was determined by ELISA.

② A, IgA alone; B, IgA combined with purified mouse sceretory component.

③ IgA concentration (μg / ml) was estimated by the concentration of IgA in stock solution divided by the actual dilution of the IgA stock in the assay.

Purified SC from mouse bile was shown to be a single band by SDS-PAGE, and was successfully assembled with either purified moIgA or moIgA in ascites. Dimer, trimer or higher polymer of moIgA combined with purified SC were demonstrated in Western blots (Fig. 2).

The effects of moIgA bound with or without SC on mouse sperm agglutination and immobilization were investigated. Monoclonal IgA PA4 was able to agglutinate and

immobilize sperm at concentrations of 430–450 μg and 110–245 μg per ml, respectively. Sperm were strongly immobilized by PA1 at concentration of 7–8 μg / ml. A relatively weak effect of PA2 on sperm immobilization was also observed. Importantly, there was no big difference in these effects between SC-bound IgA and IgA alone examined (Table 3).

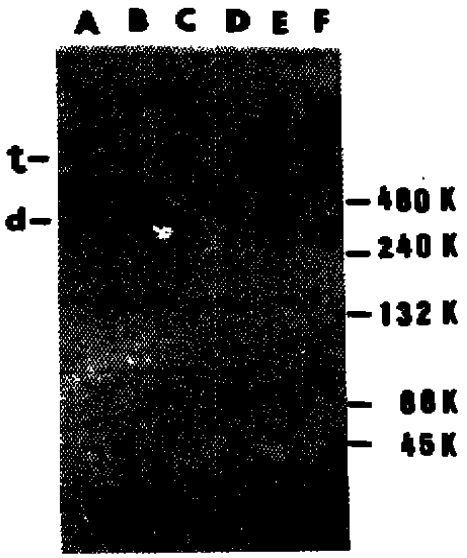

Figure 1 Western blotting of ascites containing anti-mouse LDH-C4 monoclonal IgA. Experimental conditions are described in Materials and Methods. Lanes A–F represent monoclonal IgAs PA1, PA2, PA4, PA5, PA3 and SA1, respectively. Trimer (t), dimer (d) and higher polymer forms can be seen in most monoclonals.

When moIgAs PA1, PA2 and PA5 in ascites were injected intraperitoneally into adult female mice which were superovulated by gonadotropins, and the ova and embryos in oviducts of these mice were examined at about 40–44 h after HCG injection, no significant differences of fertilization rate between all moIgA tested and paired control groups were found (Table 4).

To test the effects of moIgA with or without SC on mouse *in vitro* fertilization, the volume of IgA stock solution added for both SC-bound and-unbound groups was kept to one tenth of volume of insemination drop. Final concentrations of the moIgAs in the assay were varied from 100 to 400 μg / ml. Of seven IgA monoclonals, 3 IgAs (PA2, PA3 and PA4) were shown to inhibit *in vitro* fertilization significantly ($P<0.01$). These 3 moIgAs were all derived from PP cells. After binding with SC, their effects on fertilization rate were not obviously changed in comparison with those of IgAs alone (Table 5).

Table 4 Effects of monoclonal IgA to LDH-C4 on mouse *in vivo* fertilization

MoAb code	Nos. of embryos or eggs examined								*P* value
	Immunized group				Control group				
	A	B	C	D	A	B	C	D	
PA1	3	166	22	88.3	2	27	10	73.0	>0.01
PA2	5	117	33	78.0	3	67	15	81.7	>0.01
PA5	3	81	9	90.0	3	149	40	78.8	>0.01

A: Numbers of animals used. B: Numbers of fertilized eggs.
C: Numbers of unfertilized eggs. D: Fertilization rate (%) = B / (B+C) × 100%.

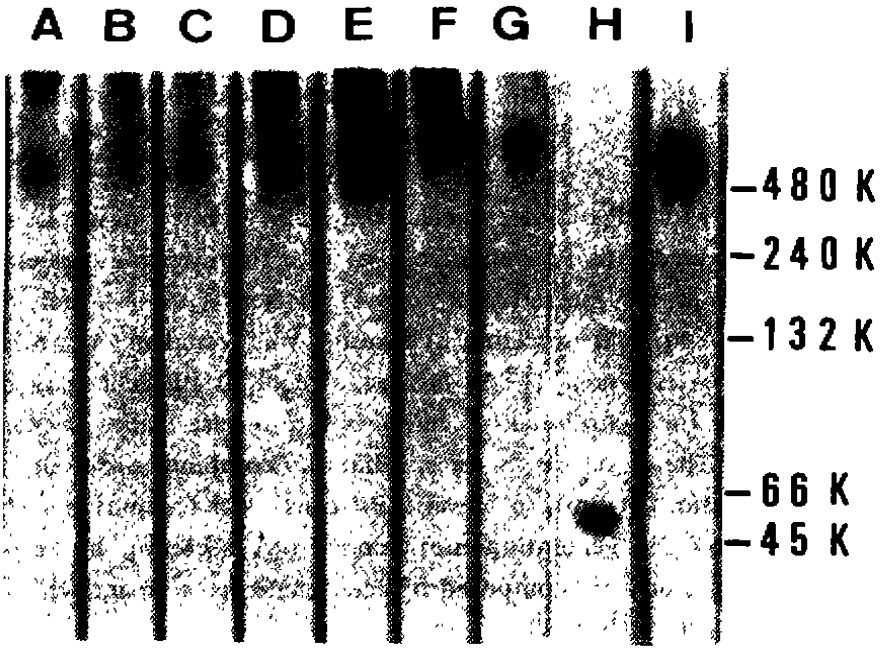

Figure 2 Patterns of moIgAs assembled with mouse purified SC are demonstrated with Western blotting. Lanes A–I represent moIgAs PA1(A), PA2(B), PA4(C), PA4(D), PA5(E), PA3(F), SA2(G) and purified PHA1(K014) (1) all bound with SC, and SC alone (H), respectively.

Table 5 Reduction of mouse *in vitro* fertilization rate by IgA to LDH–C4

MoAb① code		No. of experiments	No. of embryos or eggs observed: IgA A	IgA B	IgA C	NS1 ascites fluid A	NS1 ascites fluid B	NS1 ascites fluid C	*P* value
PA1		7	126	213	59.2	125	181	69.1	>0.01
PA2		7	136	237	57.4	153	203	75.4	<0.01
PA3	D②	6	186	355	52.4	208	286	72.7	<0.01
	E	4	53	122	43.4	102	155	65.8	<0.01
PA4	D	7	79	195	40.5	126	165	76.4	<0.01
	E	7	90	183	49.2	166	215	77.2	<0.01
PA5	D	2	73	85	85.9	49	66	74.2	>0.01
	E	4	83	123	67.5	78	108	72.2	>0.01
SA1		2	61	84	72.6	36	46	78.3	>0.01
SA2		5	76	136	55.9	101	138	73.2	>0.01

① Final concentrations of IgA in the insemination drop ranged from 100–400 μg / ml.

② D, antibody alone; E, the antibody assembled with mouse purified SC; A = fertilized; B = examined; C = fertilization rate(%).

4 DISCUSSION

In some experimental models, genital tracts have been shown to be a part of the common mucosal immune system (Mesteckey and Mcghee, 1987). The humoral im-

mune response to sperm in genital and digestive tracts can be stimulated by oral or intragastrointestinal immunization of mice with either sperm antigen extracts or whole sperm (Chen *et al.*, 1991). However, controversial results on reduction of fertility in mice were reported by different groups after the mice were intragastrointestinally immunized with sperm (Curtis and Ryan, 1982; Parr and Parr, 1986). In the present study, cloned hybriodomas secreting antibodies to LDH-C4 were prepared by fusion of NS-1 myeloma cells with PP or spleen cells from Balb/c mice immunized with purified murine LDH-C4 injected into PP submucosa or into the intertinal lumen (Table 1 and 2). The proportion of IgA-secreting B cells in PP seems to be much higher than in spleen. Surprisingly, no IgG-secreting hybridoma was found in the present study (Table 2). Recently, Weltzin *et al.*(1989) also reported that PP cells may be better than spleen cells for generating hybridomas secreting IgA to mouse mammary tumor virus and reovirus type 1. These results suggest that a combined use of intraintestinal immunization and fusion of antigen specific PP cells used for parent cells may be the most effective method for generating monoclonal IgA secreting hybridomas. Most moIgAs in the present study are composed of heterogeneous isoforms which were demonstrated by Western blotting (Fig. 1).

According to the results of recent studies, the first domain of SC binds noncovalently to the Cα3 domain of polymer IgA, and two cysteine residues (in the form of a labile disulfide bond) of the fifth domain of SC are involved in the formation of two disulfide bridges with cysteine residues in two Cα2 domains within a single monomeric subuint of polymer IgA (Mestecky, 1988; Kerr, 1990). The *in vitro* binding of murine bile SC to polymer IgA against LDH-C4 by the method described above is likely based on these molecular interactions.

The pattern of Western blotting (Fig. 1) clearly showed that variation exists in the molecular weight of the same polymer IgA produced by different hybridomas. This phenomenon also appeared in monoclonal IgA directed against virus proteins (Weltzin *et al.*, 1989). It may be due to the variable glycosylation of IgA during IgA synthesis within the B cell.

It is well known that complement is involved in human and mouse IgG and IgM mediated sperm immobilization (Anderson *et al.*, 1987; Isojima and Koyama, 1988). The activation of complement by IgA remains a cotroversial subject (Kerr, 1990). In the present study, monoclonal IgA PA1 showed a high titer for mouse sperm immobilization, and PA2 and PA4 at high concentration are able to immobilize sperm (Table 3). That IgA participates in a complement mediated sperm immobilizating is supported by a recent study demonstrating that human IgA1 initiates complement-mediated killing of *Neisseria menigitidis* (Jarvis and Griffis, 1989). In addition, it is reported that murine monoclonal IgA to group B streptococci enhances phagocytosis by rat peritoneal macrophages *in vitro* in the presence of complement (Bohnsack *et al.*, 1989).

When three moIgAs (PA1, PA2 and PA5) were intraperitoneally injected into adult female mice, no significant reduction of fertilization rate was observed. A possible explanation is that fertilization rather than pregnancy is the endpoint in the present study. This result is similar to a recent report that the *in vivo* fertilization rate of male but not female mice immunized with LDH-C4 was impaired (Mahi-Brown *et al.*, 1990). Another possible reason is that a sufficient concentration of the moIgA in the female genital tracts for fertilization inhibition is not obtained when the female mice are passively immunized by intraperitoneal injection (Saling and Waibel, 1985).

Distinct biological function of various moIgAs are found in sperm agglutination and immobilization as well as in *in vitro* fertilization. It may be caused by the differences in moIgA-corresponding antigenic epitopes which are not functionally equal for the LDH-C4 molecule.

There are no significant changes in the effects of moIgA on sperm agglutination, sperm immobilization and *in vitro* fertilization when assembled with SC (Table 3 and 5). These results suggest that local mucosal IgA (secretory IgA) to sperm may play an important role in immunological infertility, and that the stimulation and maintenance of sufficient production of secretory IgA in genital tracts might be considered as one of key factors in development of antisperm contraceptive vaccine.

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抗小鼠精子特有乳酸脱氢酶 C4 的与分泌片装配或未装配的单克隆抗体 IgA 的生物学功能

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摘要 为了测定抗精子 IgA 在免疫不育和抗精子避孕疫苗研制方面的生物学作用, 用肠道内免疫的方法制备了一组抗乳酸脱氢酶 C4(LDH-C4)的单克隆 IgA 抗体(moIgA)。以免疫印迹证实了它们的异质同形体。大部分 moIgA(PA1-PA5)是用肠道内免疫和以派依尔氏淋巴细胞作为亲本细胞进行融合来获得的。在豚鼠血清补体存在的情况下, 小鼠精子可以被 moIgA PA1、PA2 和 PA4 所制动。高浓度 PA4 和 PA5 可凝集小鼠精子。小鼠体外受精率可被 3 个 moIgA(PA2、PA3 和 PA4)显著降低, 但用 PA1、PA2 和 PA5 被动免疫之后, 小鼠体内受精无明显变化。纯化的小鼠胆汁分泌片可同纯化的 moIgA 或腹水中的 moIgA 在体外组装起来。同分泌片结合之后, moIgA 对精子的制动、凝集和体外受精无明显变化。这些研究结果提供了抗 LDH-C4 的 moIgA 和分泌性 IgA 对精子功能和体外受精的生物学作用的直接证据, 在免疫不育的防治, 避孕疫苗的研制以及性传播疾病的防治方面均有一定的指导意义。

关键词 单克隆 IgA, 乳酸脱氢酶 C4, 精子制动和凝集, 体外受精, 分泌片

单克隆抗体, 免疫球蛋白(Ig), 乳酸脱氢酶,

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